SPECIATION IN FISHES OF THE GENERA *CYPRINODON* AND *EMPETRICHTHYS*, INHABITING THE DEATH VALLEY REGION [1]

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INTRODUCTION

During the Pleistocene history of the desert basins of western North America, particularly in the Great Basin, block faulting produced dozens of independent valleys separated by precipitous mountain ranges. In Pluvial [2] periods many of these valleys held lakes and large streams, but the desiccation of postglacial times disrupted the drainages between, as well as within, the once continuous basins. Isolated populations of animals and plants resulted, and the Great Basin has become a land of relicts.

The Death Valley region, lying in the southwestern extension of the Great Basin, provides an excellent example of such disconnected drainages and faunas. During late Pleistocene time, an integrated river system spread over this desert region to unite the four now isolated basins of what may be called the Death Valley system (Map 1).

Although only a limited fish fauna inhabits this area, it is rich in material for the student of evolution and is highly endemic. The living fishes comprise 3 families, 5 genera, 10 species and about 24 kinds including subspecies. One genus, *Empetrichthys,* is endemic, and nine species are confined to the system. High endemism on the species level suggests a Wisconsin or pre-Wisconsin origin of the fauna.

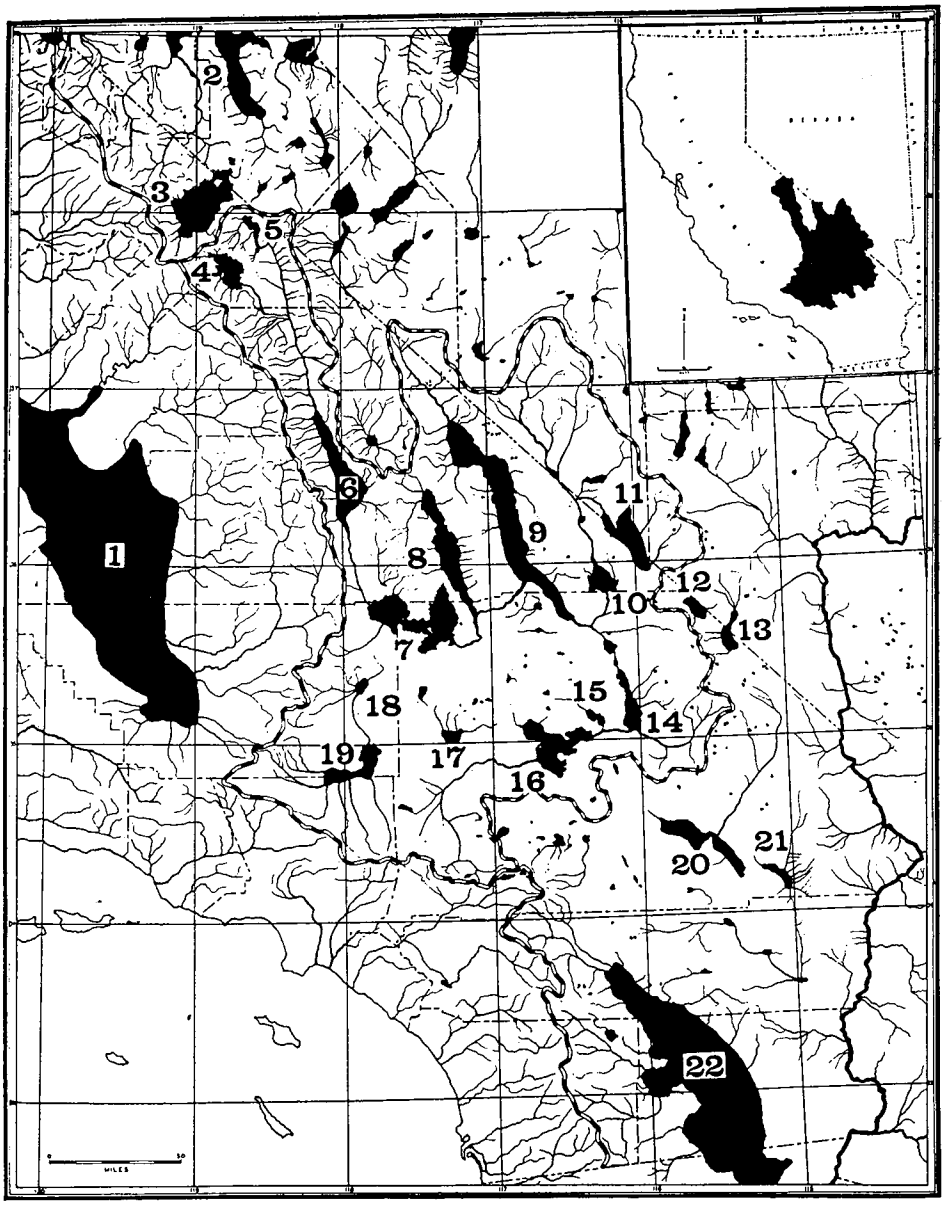
[1] This paper is summarized from the lengthy original (Miller, 1948) with modifications and additions.

[2] The term Pluvial refers to the two or more stages, commonly regarded as of late Pleistocene (Wisconsin) age, when desert basins in western North America held lakes that have left definite records of their existence in the form of shore features.

The present account is limited to a discussion of speciation in the six species represented by the cyprinodontid genera *Cyprinodon* and *Empetrichthys,* which were recently treated in detail (Miller, 1948).

DIFFERENTIATION

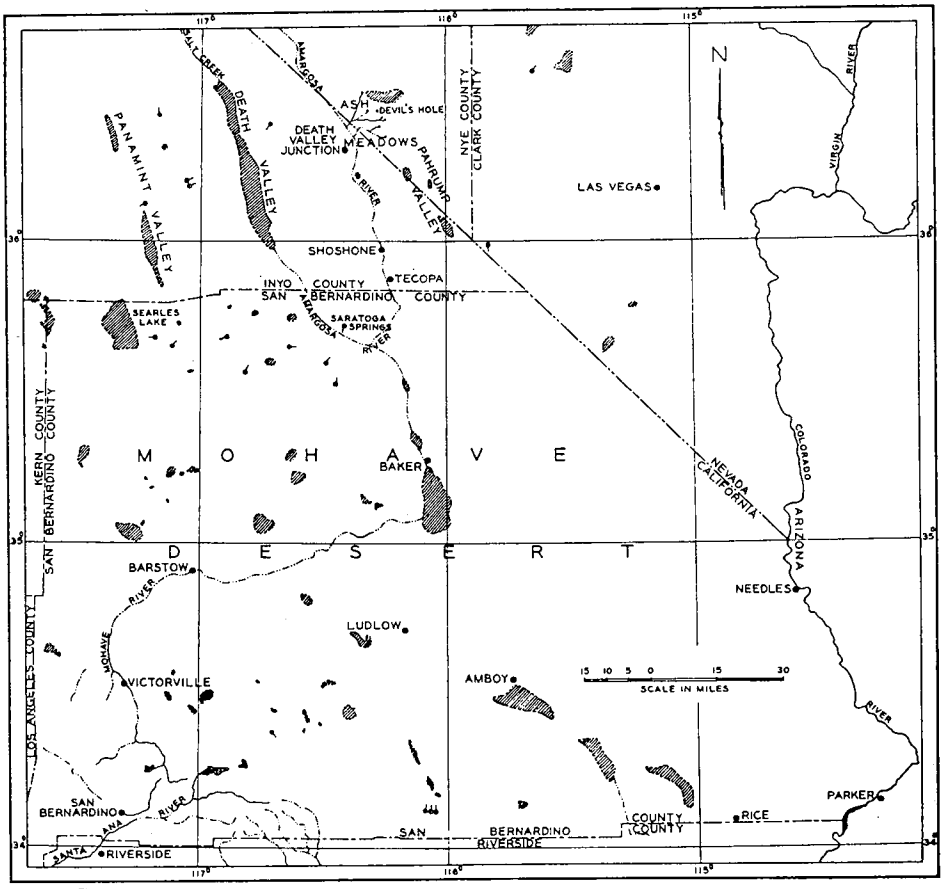
The genus *Cyprinodon,* which comprises small fishes that tolerate a wide variety of physical and chemical conditions, is represented by four distinct species: *C. radiosus* in Owens Valley, along the eastern base of the Sierra Nevada Range; *C. salinus* in Salt Creek, on the floor of Death Valley; *C. nevadensis* in the Amargosa River drainage system, from the south end of Death Valley to just across the California-Nevada border; and *C. diabolis* of Devil's Hole, an isolated spring in Ash Meadows, Nevada (Map 2). Each of these species is sharply differentated, and their characters run nearly the entire gamut of known variation in *Cyprinodon,* a genus found also in eastern and southern United States and southward to northern South America. All four are tied together, however, by a basic pattern of scale structure that distinguishes the species of the Death Valley system from those elsewhere (Miller, 1948, Pl. 2). This harmony in fundamental scale pattern is a striking confirmation of the interrelationship of these species, and is convincing zoological evidence to support the geological data that the waters of the Death Valley system were once continuous (Map 1). Differentiation in *C. nevadensis,* of the Amargosa River drainage, has been sufficient for the naming of 6 subspecies and the



MAP 1. Maximum extent of late Pleistocene waters in the southern Great Basin and adjoining regions. The wider boundary line marks the separation between coastal and interior or Colorado River drainages. The narrower line indicates the outline of the Death Valley system and enclosed basins (see inset). The authorities for the Pluvial lakes are cited by Miller (1946) and by Hubbs and Miller (1948).

1. Lake Tulare
2. Walker Lake arm of Lake Lahontan
3. Lake Russell [1]
4. Lake Long Valley
5. Lake Adobe
6. Lake Owens
7. Lake Searles
8. Lake Panamint
9. Lake Manly
10. Lake Tecopa
11. Lake Pahrump
12. Lake Mesquite
13. Lake Ivanpah
14. Lake Mohave
15. Little Lake Mohave
16. Lake Manix
17. Lake Harper
18. Lake Kane
19. Lake Thompson
20. Lake Amboy
21. Lake Ward
22. Lake LeConte

[1] Named by Putnam (1949)



MAP 2. Sketch map of southeastern California and adjacent areas, showing the shrunken remnants of Pluvial waters and localities mentioned in the text.

recognition of numerous races.[3] Isolated springs and a few disconnected surface flows are all that remain of this river, but during periods of heavy rain in the headwaters, Amargosa River comes to life as it pours its flood waters onto the great Death Valley salt flat—the remnant of Pluvial Lake Manly. Even during such flood stages, however, there is no evidence that the many fish populations intermingle.

The genus *Empetrichthys,* restricted to warm springs and their outflows, is represented by two species: *E. merriami,* inhabiting Ash Meadows of the Amargosa River drainage, and *E. latos,* known only from Pahrump Valley (Map 2). The latter species has differentiated into 3 subspecies. These localities, both in southern Nye County, Nevada, are isolated by a low aluvial divide. Pahrump Valley is a wholly enclosed basin, whereas during high water stages the outflows of the springs in Ash Meadows join Amargosa River, which also contains water at such times. Until recently, *Empetrichthys* was not known to have any close relative, and at one time it was thought to be related to *Orestias,* a genus peculiar to Lake Titicaca and other elevated waters of

[3] Following rather general usage in ichthyology, the term race is applied to the next systematic category below the subspecies; it does not ordinarily receive nomenclatorial recognition.

the Andes. Representatives of this remarkable genus are very rare in Ash Meadows, for only 22 specimens have been secured over a six-year period, but they are rather common in Pahrump Valley. In Ash Meadows *Empetrichthys* is associated with *Cyprinodon nevadensis* and the cyprinid, *Rhinichthys osculus,* but in Pahrump Valley it constitutes the sole native fish life. The comparative abundance of *E. latos* there is perhaps due largely to lack of competition. There may be some local differentiation in the populations of *E. merriami* inhabiting the various springs in Ash Meadows, but if so the material collected to date is insufficient to determine whether the observed variations are significant.

Factors Affecting Speciation Rates

There is evidence that at least 4 factors play an important role in modifying rates of evolution in these desert fishes.

POPULATION SIZE

In very small, closely inbreeding populations, the probability of accidental survival of particular genes or gene combinations is greatly increased. Sewall Wright has ably demonstrated the theoretical basis for this chance fixation of characters which, in the absence of interspecific competition, may be more important than selection in the evolution of such isolates. The chances for recessive mutations to become homozygous are better than they are in large populations of more widespread distribution. The determining factor is the size of the minimum effective breeding population. In some of the spring-inhibiting fish in the Death Valley region, the breeding population frequently drops to a minimum of several hundred individuals or considerably less. The most striking example is afforded by *Cyprinodon diabolis,* a dwarf species confined to a single hot-spring hole where the entire population occupies a habitat about 20 feet long, 8 feet wide, and a few inches to a few feet deep. Since every fish is in view at one time, reliable estimates of the total population are possible. The number of individuals of this species fluctuates between about 50 and 400. At times the minimum effective breeding population may thus fall below 50 individuals. No doubt the Sewall Wright effect has been a very significant factor in the evolution of this remarkable species, which is one of the most distinctive in the genus.

DURATION OF ISOLATION

The postpluvial disruption of rivers, lakes, and springs in the arid West reulted in the separation of fish populations into isolated stocks, some of which have subsequently differentiated into distinct species and subspecies. Others have attained only the racial level of differentiation. It may be stated as a general rule, for desert fishes, that a positive correlation exists between duration of isolation and extent of differentiation (Hubbs and Miller, 1948). In the Death Valley region, those populations of *Cyprinodon* estimated to have been isolated since early Wisconsin time are now distinct species, sharply differentiated from their nearest relatives. If placed in the hands of a systematist unfamiliar with the group, they might readily be regarded as comprising two or three genera. On the other hand, the close relationship among the six subspecies and the many races of *Cyprinodon nevadensis* in the basin of Amargosa River indicates recency of isolation. Geologists believe that the Amargosa may have been a continuous river as recently as a few centuries ago, at most a few thousand years ago. Nevertheless, we cannot be sure that postpluvial disruption of drainages accounts for all such speciation, since permament rivers today in other parts of the West also contain endemic forms.

The duration of time since Pahrump Valley and Ash Meadows (of the Amargosa River basin) have been isolated from each other cannot be reliably estimated in the absence of detailed studies of the past hydrography of these two regions. The isolation has been of sufficient dura-

tion to allow for the differentiation of a species of *Empetrichthys* in each basin. In Pahrump Valley, the evolution of 3 subspecies of *E. latos* in isolated springs suggests that the lake which formerly covered the valley floor was of late Pleistocene age, and the physiographical evidence supports this view. Two of the springs are less than 1 mile apart and the third is only 7 miles distant.

ECOLOGICAL CONDITIONS

Ecological conditions in desert springs are believed to play an important role in hastening evolution. These springs are very diverse, physically and chemically, and their contained fish life presumably has become highly adapted to this great variety of conditions (Sumner and Lanham, 1942). It may be assumed that the conditions in each spring and creek in some way influence the development of the remnant populations. What effect these chemical and physical characteristics may have in modifying the characters of the fishes is known only for two striking features of springs and creeks, namely, high temperature and salinity. Some of the springs in the Death Valley region are remarkably high in boron content, but no investigation has been undertaken to determine what effect, if any, this element may have on the fishes.

Much evidence has been accumulated to demonstrate that high temperature plays an important role in accelerating the rate of speciation (Plough, 1942, and other authors in same symposium). The repetition of certain striking trends in warm-spring fishes is a significant expression of this relationship: (1) the number of meristic segments is generally reduced; (2) the position of the dorsal fin is often more posterior; (3) the head, eye and other anterior parts of the body are enlarged; (4) the size is very frequently reduced; (5) the pelvic fins and rays of *Cyprinodon* tend to be reduced in size and number, or the fins may be entirely lacking; and other modifications have been observed. That some or all of these trends are genetically determined, at least in part, is indicated by preliminary breeding experiments and by the more carefully controlled work on *Fundulus* by Gabriel (1944). These show that F_1 generations retain essentially the same meristic characters found in the natural stocks. A few exceptions, however, indicate that altered environment, as well as heredity, is a factor determining the number of parts.

Observations and experiments have shown that there is a very general correlation between temperature and certain meristic characters in fishes. Thus an increase in temperature usually is associated with a decrease in number of fin rays, scales or vertebrae, and, vice versa, in cooler waters the number of segments is typically increased. This picture is oversimplified, however and citing temperature as the only, or major, causal factor is treading on uncertain ground. Many other factors undoubtedly operate in conjunction with accelerated temperature, but what these factors may be and how they affect the organism remains to be determined.

Among certain subspecies and races of *Cyprinodon nevadensis*, a striking lack of correlation between temperature and meristic characters may be demonstrated (table 1). For example, in comparing *pectoralis* with the race of *mionectes* inhabiting the source pool of Point of Rocks Spring (columns 2 and 4, table 1), it is seen that the meristic characters are consistently and significantly higher for *pectoralis* even though it inhabits the warmer of the two springs. This suggests that high temperature may have accelerated the production of mutations and that the random inheritance of these changes may have produced the variant structure of such populations. Plough (1942: 14–15) has shown that high temperature, as well as temperature shocks, greatly increases the mutation rate in *Drosophila*.

Although no clear-cut correlation between temperature and meristic characters can be demonstrated by comparing distinct subspecies or races within one

area or between two regions, a general correlation of this nature is evident. This is epecially true when populations confined to the limits of a single spring and its outlet are considered. Here one finds a significant positive correlation between temperature and certain meristic characters. This correlation is particularly well illustrated by the number of scales around the body (fig. 1). In the samples from the spring outlets, which are consistently cooler than the source springs, the body scales are significantly increased when compared with the fish from the springs. In each complex of spring source and outlet there appears to be a distinct genetic stock, the members of which respond to temperature differences in the same way. Although the spring sources may have identical or closely approximated temperatures, the average number of body scales is often not the same. For example, the temperature of Hidden Spring is nearly identical with that of Eagle or of Deep Spring, but the average number of body scales differs significantly from the samples from either of those springs (fig. 1).

Increasing salinity seems to have about the same effect on speciation as decreasing temperature, namely an increase in certain meristic parts. In *Cyprinodon salinus,* the species that inhabits the briny waters of Salt Creek on the floor of Death Valley, the scales are smaller than in any other known species of the genus. In the Death Valley race of *Cyprinodon nevadensis amargosae,* found in the waters of Amargosa River, which are less saline than those of Salt Creek, the scale counts

TABLE 1. *Correlation between certain meristic characters and temperature in five warm-spring populations of* Cyprinodon nevadensis

Means are given above, and number of specimens and standard error of the mean below in parentheses.

Subspecies	*shoshone*	*mionectes*	*mionectes*	*pectoralis*	*calidae*
Locality[1]	A	B	C	D	E
Temperature	30° C.	31° C.	31° C.	33° C.	37° C.
Anal rays	10.07	9.62	9.88	10.03	10.08
	(133, ±.04)	(103, ±.05)	(67, ±.05)	(100, ±.04)	(100, ±.04)
Pectoral rays	15.99	15.68	15.54	17.10	15.88
	(214, ±.04)	(193, ±.05)	(233, ±.04)	(200, ±.04)	(200, ±.05)
Pelvic rays[2]	4.98	2.99	5.01	5.12	5.33
	(258, —)	(206, —)	(234, —)	(200, —)	(200, —)
Lateral scales	25.72	24.59	25.40	25.42	25.58
	(116, ±.05)	(95, ±.06)	(67, ±.07)	(90, ±.06)	(100, ±.05)
Dorsal to pelvic scales	9.39	8.75	9.14	9.79	9.35
	(48, ±.08)	(36, ±.07)	(49, ±.08)	(49, ±.08)	(94, ±.06)
Dorsal to anal scales	8.88	8.50	8.86	8.94	8.94
	(50, ±.06)	(50, ±.07)	(50, ±.07)	(50, ±.05)	(100, ±.05)
Predorsal scales	18.50	17.96	17.82	17.91	17.71
	(111, ±.11)	(50, ±.13)	(67, ±.12)	(90, ±.12)	(99, ±.10)
Caudal peduncle scales[2]	15.64	13.00	14.34	15.44	15.71
	(84, —)	(95, —)	(67, —)	(89, —)	(100, —)
Body scales	23.25	20.44	23.10	24.75	23.06
	(84, ±.17)	(91, ±.12)	(67, ±.22)	(89, ±.19)	(100, ±.10)

[1] A = Outlet, Shoshone Spring
B = Source, Point of Rocks Spring
C = Big Spring
D = Lovell's Spring
E = Outlet, South Tecopa Hot Spring
A and E lie about 8 miles apart in the Middle Amargosa River basin, California; B, C, and D are 1½ to 9 miles apart in Ash Meadows, Nye County, Nevada (Map 2).

[2] The standard error of the mean was not calculated for these data as they present a markedly skewed curve and the derivation of this statistic assumes a normal frequency curve (Miller, 1948: 15).

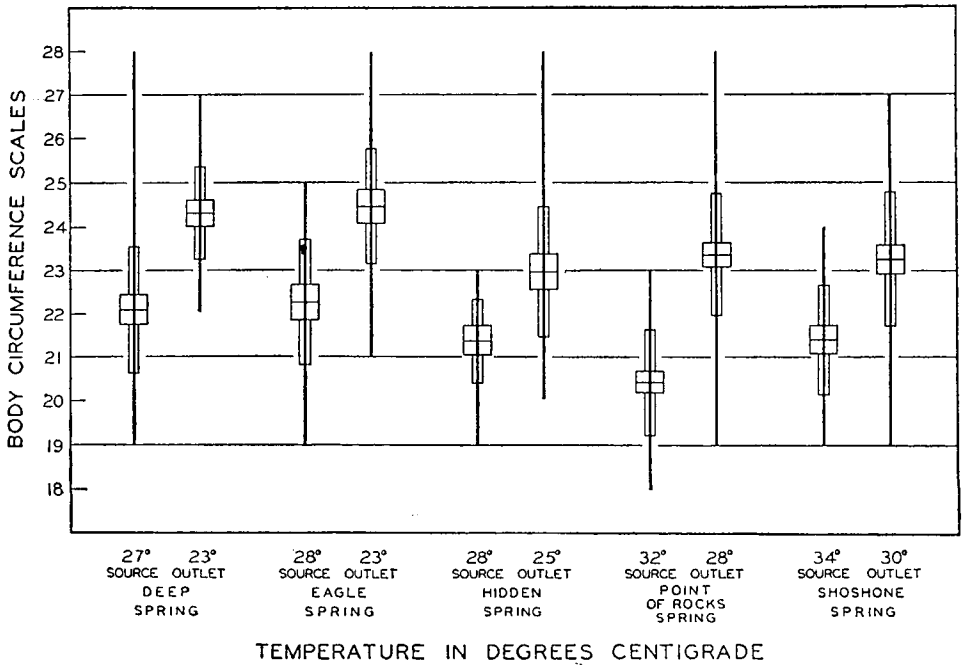


FIG. 1. Correlation between body circumference scales and temperature in certain populations of *Cyprinodon nevadensis* from the Amargosa River basin.

average higher than in any other form of *C. nevadensis*. In the spring-fed lakes at Saratoga Springs, Death Valley, where *C. nevadensis nevadensis* spawns, the waters are only moderately salty and the average number of scales around the body is lower than it is at either of the preceding localities. A direct, environmental influence is indicated, but it is thought that the observed differences are due in part to genetic adaptations that parallel those due to the direct effect of the surroundings.

NUMBER OF GENERATIONS PER YEAR

Speciation is evidently a particularly rapid and extensive process in warm springs. The role that high temperature may play in hastening this process already has been discussed. A secondary and highly significant effect of raised temperatures is the increase in number of generations that are produced each year by the warm-spring fishes.

The numerous warm springs of the Death Valley region have temperatures that vary from about 22° to 43° C. (72° to 109° F.), although fish do not tolerate temperatures higher than 40° C. (104° F.). Individually the temperature of each spring fluctuates very narrowly over the years (Miller, 1948: 130). There is evidence that *Cyprinodon* (and presumably *Empetrichthys* also) spawns every month of the year in the warmer springs (those 28° C. or higher), whereas the limits of the breeding period for the stream populations are approximately from April to October. In contrast to the 2 (or perhaps 3) generations produced per year by the stream stocks, as many as 8 or 10 generations may be the annual production for the warm-spring fish. Experimental studies have shown that *Cyprinodon nevadensis* may reach maturity and produce young when only 2 months old. Since these fish were born and developed in much cooler water than that of their native warm springs, it seems safe to as-

sume that the warm-spring fish are capable of breeding in nature when only 1 month or 6 weeks old. This production of numerous generations each year may greatly accelerate the time rate of evolution of such populations.

The isolation of small, breeding populations that are subjected to an accelerated mutation rate and an increase in number of generations, due to high temperature, are believed to result in the striking differentiation exhibited by such species as *Cyprinodon diabolis*.

Experimental Work

The rearing of three subspecies of *Cyprinodon nevadensis* in concrete pools, under physical and chemical conditions unlike those in their natural habitats, has led to some interesting results that need further checking. With only few exceptions, analysis of meristic characters of the F_1 has demonstrated that these generations retain the characters of their respective subspecies in nature. In general, the average values for the first generations of the three subspecies lie in the same direction as do the values for the natural stocks of these subspecies. If the subspecies in nature do not differ significantly in a particular count, the values for the F_1 stock likewise are not significantly different.

Interspecific, as well as intraspecific, hybridization was carried out. When species of *Cyprinodon* were crossed, lowered fertility generally resulted, particularly in certain reciprocal crosses. Attempts to mate males of *Cyprinodon salinus* with females of *Cyprinodon macularius* (a species confined to the lower Colorado River basin) failed. Mating females of *salinus* with males of *nevadensis* resulted in several generations of hybrids, but comparatively few fish were produced. The most successful interspecific matings took place between *nevadensis* and *macularius*, but the success of some of these combinatinons varied with the reciprocal crossings. For example, when males of *C. macularius* were crossed with females of *C. nevadensis amargosae*, only moderate success was obtained, but when males of *amargosae* were hybridized with females of *macularius*, very good results ensued. Some of the variations in productivity were probably influenced by differences such as size, temperature, and exposure, in the various pools used in the experiments.

In the hybridization between subspecies of *C. nevadensis*, best results were obtained when the two spring forms (*nevadensis* and *shoshone*) were *crossed*. The two other combinations, *amargosae* × *nevadensis* and *amargosae* × *shoshone*, involved one parent form (*amargosae*) that develops in nature in saline water. . The experimental pools contained fresh water. Whether this factor had an important bearing on the poor results when *amargosae* was used as one parent, or whether *nevadensis* and *shoshone* are more closely related to each other than either one is to *amargosae*, is problematical.

Paleontological Evidence

Although the paleontological data on fishes from western United States are woefully incomplete, a study of the few available Miocene fossils reveals a fauna quite unlike that now inhabiting the region. It is therefore assumed that the recent fish fauna is not much older than Pliocene. Pleistocene fish remains should be of great importance in working out the more recent history of the fish fauna, but the few specimens collected thus far have been largely fragmentary.

The occurrence of 3 species of *Fundulus* in Death Valley and the Mohave Desert in late Pliocene or early Pleistocene time, and of a fossil *Cyprinodon* in Death Valley during the same period (Miller, 1945), is of significance in attempting to elucidate the origin of the cyprinodontid fauna. *Fundulus* and *Cyprinodon* are widespread today in regions of low altitude and are particularly abundant near and along coastal areas. In particular, the presence of *Fundulus* suggests a lowland connection to the sea from the southwest, as the only living members of this genus in the

Pacific drainage are now found from Monterey Bay, California, nearly to the tip of Lower California. *Cyprinodon* clearly came in from the southeast, in the direction of the Colorado River (Miller, 1946).

The endemic genus *Empetrichthys,* whose nearest living relative is *Crenichthys* of eastern Nevada, is evidently an old (pre-Pleistocene?) relict. Both genera were presumably derived from *Fundulus,* and *Empetrichthys* may have descended from a species similar to *F. curryi,* one of the fossils from Death Valley (Miller, 1945: fig. 1).

Summary

Speciation in the cyprinodontid fishes inhabiting the Death Valley region is correlated with the disruption of waters since late Pleistocene times, with population size, with the number of generations produced each year, and with physical and chemical conditions of the remnant springs and creeks. There are two living genera, *Cyprinodon* with 4 species, one of which has 6 subspecies, and *Empetrichthys* with 2 species of which one has 3 subspecies. All of the species and subspecies are endemic, as is *Empetrichthys* which is evidently an ancient relict. Three fossil representatives of *Fundulus,* probably of late Pliocene or early Pleistocene age, indicate a lowland connection to the southwest. One of the species may have been near the ancestral stock from which *Empetrichthys* was derived. The *Cyprinodon* reached Death Valley from the southeast, by way of the Colorado River basin or its antecedent. Most of the subspecies of *Cyprinodon nevadensis* show racial differentiation, particularly *C. n. mionectes* of Ash Meadows, Nevada. High temperature is believed to have accelerated the time rate of evolution in warm-spring populations by increasing the mutation rate and the number of generations produced each year. Many of the local subspecies and races have very small ranges, which may be closely approximated. One unique species, *Cyprinodon diabolis,* is confined to a single small spring in which its total numbers fluctuate from about 50 to 400 individuals. Experimental studies have demonstrated that F_1 generations of pure stocks of three subspecies of *Cyprinodon nevadensis,* reared under environmental conditions greatly altered from those of their natural habitat, maintained essentially the same meristic characters as are found in the natural populations. A few exceptions, however, indicate that altered environment, as well as heredity, is a factor in determining the number of segments.

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